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Comparative Analysis of Two Commercial Phenotypic Assays for Drug Susceptibility Testing of Human Immunodeficiency Virus Type 1

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Human immunodeficiency virus type 1 (HIV-1) isolates from 50 plasma specimens were analyzed for phenotypic susceptibility to licensed reverse transcriptase inhibitors and protease inhibitors by the Antivirogram and PhenoSense HIV assays. Twenty of these specimens were from recently seroconverted drug-naïve persons, and 30 were from patients who were the sources of occupational exposures to HIV-1; 16 of the specimens in the latter group were from drug-experienced patients. The phenotypic results of the Antivirogram and PhenoSense HIV assays were categorized as sensitive or reduced susceptibility on the basis of the cutoff values established by the manufacturers of each assay. Data for 12 to 15 drugs were available by both assays for 38 specimens and represented a total of 529 pairs of results. The two data sets had a 91.5% concordance by phenotypic category. The discordant results (n = 45) were distributed randomly among 26 specimens and included 28 results (62.2%) which were within a twofold difference of the assay cutoff values. None of the discordant results were associated with primary resistance mutations that predicted high-level (>20-fold) resistance. Discordant results were distributed equally among specimens from drug-experienced and drug-naïve individuals and were slightly higher for protease inhibitors than for nonnucleoside reverse transcriptase inhibitors or nucleoside reverse transcriptase inhibitors. The findings of the present study demonstrate that the results of the Antivirogram and PhenoSense HIV assays correlate well, despite the use of different testing strategies.

Treatment of human immunodeficiency virus type 1 (HIV-1)-infected persons with antiretroviral drugs selected on the basis of resistance testing has been associated with improved virologic responses (7). HIV-1 resistance testing is recommended to guide the choice of new drug regimens after the first or multiple treatment failures (7). To date, several phenotypic and genotypic assays have been developed and are being used to monitor drug resistance (6). Genotypic assays are based on the detection of resistance-related mutations and provide indirect evidence of resistance (2, 3, 17, 19). Phenotypic assays measure the ability of the virus to replicate in the presence of a drug and thus provide a direct measurement of drug susceptibility. Comparison of the drug concentrations required to inhibit 50% (IC_{50}) of patient virus replication with the IC_{50} for a drug-susceptible reference virus is used to measure reductions in drug susceptibility. Conventional phenotypic assays require the use of HIV-1 isolates, which can be obtained by culturing peripheral blood mononuclear cells or plasma. The need for virus isolation adds labor, time, and cost (2, 9, 12). New phenotypic assays circumvent the requirement for virus isolation by generating recombinant viruses from patient-derived protease (PR) and reverse transcriptase (RT) sequences and proviral constructs from which the sequences for PR and RT are deleted. The recombinant viruses are then used for drug susceptibility testing in standardized assays (5, 10, 16, 18). The Antivirogram and PhenoSense HIV assays are two commercially available recombinant virus-based assays developed by Virco (Mechelen, Belgium, and Cambridge, United Kingdom) and ViroLogic Inc. (South San Francisco, Calif.), respectively (5, 10). These assays use different strategies to generate recombinant viruses and measure drug susceptibility.

As drug resistance testing becomes an integral part of patient management, an evaluation of the correlation between the results of the available drug resistance testing assays will be needed. This issue is of particular importance for phenotypic testing because of the complexities of the assays and the use of different testing strategies by commercial assay providers. Since no information on the concordance between the results of two commercial phenotyping assays, the Antivirogram and PhenoSense HIV assays, is available, we analyzed a set of plasma specimens from HIV-1-infected persons by both assays and compared the results.

MATERIALS AND METHODS

Study design and plasma specimens. A collection of 50 plasma specimens from HIV-1-infected persons was selected at the Centers for Disease Control and Prevention (CDC). These specimens were aliquoted at CDC into two sets of 500 μl each and were given different labels. In collaboration with both Virco and ViroLogic Inc., one set of specimens was shipped to Virco for analysis by the Antivirogram assay, while the second set was shipped to ViroLogic for analysis by the PhenoSense HIV assay. Test results from each company were provided to CDC for analysis. Of the 50 specimens, 20 were from recently seroconverted drug-naïve persons (21) and 30 specimens were from patients who were the sources of occupational exposures; 16 of the specimens in the latter group were

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TABLE 1. Comparison of concordant and discordant drug susceptibility testing results for 38 HIV-1 plasma specimens analyzed by Antivirogram and PhenoSense HIV assays for each drug and drug class

Result	No. of observations[a]															
	NRTIs or NtRTI							NNRTIs			PIs					Total
	AZT	3TC	d4T	ddI	ddC	ABC	ADV	NVP	DLV	EFV	SQV	IDV	RTV	NFV	AMP	
Total	37	38	38	37	38	38	20	37	36	38	38	38	38	38	20	529
Concordant																
Sensitive	32	30	36	37	35	33	19	29	30	32	31	35	30	29	19	457
Reduced susceptibility	3	5	0	0	0	0	0	4	3	3	1	1	2	4	1	27
Total concordant	35	35	36	37	35	33	19	33	33	35	32	36	32	33	20	484
Discordant																
AV-r and PS-s[b]	0	1	1	0	3	0	0	1	0	0	5	2	2	2	0	17
PS-r and AV-s[c]	2	2	1	0	0	5	1	3	3	3	1	0	4	3	0	28
Total discordant	2	3	2	0	3	5	1	4	3	3	6	2	6	5	0	45

[a] Drug abbreviations: AZT, zidovudine; 3TC, lamivudine; d4T, stavudine; ddI, didanosine; ddC, zalcitabine; ABC, abacavir; ADV, adefovir; NVP, nevirapine; DLV, delavirdine; EFV, efavirenz; SQV, saquinavir; IDV, indinavir; RTV, ritonavir; NFV, nelfinavir; AMP, amprenavir.
[b] Reduced susceptibility by the Antivirogram assay but sensitive by the PhenoSense HIV assay.
[c] Reduced susceptibility by the PhenoSense HIV assay but sensitive by the Antivirogram assay.

from drug-experienced persons. The history of treatment with antiretroviral drugs was available for all patients but was not revealed to the testing laboratories.

Phenotypic drug resistance analysis. Specimens were tested for resistance to 12 to 15 drugs by the Antivirogram and PhenoSense HIV assays. The drugs included six nucleoside RT inhibitors (NRTIs; zidovudine, lamivudine, zalcitabine, stavudine, didanosine, and abacavir), one nucleotide RT inhibitor (NtRTI; adefovir), three non-NRTIs (NNRTIs; nevirapine, delavirdine, and efavirenz), and five PR inhibitors (PIs; indinavir, nelfinavir, saquinavir, ritonavir, and amprenavir).

The Antivirogram assay was performed at Virco by the recombinant virus assay approach described by Hertogs et al. (5), with modifications, as described elsewhere (R. J. Powells, K. Hertogs, S. Kemp, S. Bloor, K. Acker, J. Hansen, W. Beukeleer, C. Roelant, B. Larder, and P. Stoffels, Abstr. 2nd Int. Workshop HIV Drug Resist. Treatment Strategies, abstr. 51, 1998). Briefly, the PR- and RT-coding sequences were amplified from patient-derived viral RNA with HIV-1-specific primers. After homologous recombination of amplicons into a proviral clone from which the coding sequences for PR and RT were deleted, the resulting recombinant viruses were harvested, titrated, and used for in vitro testing of susceptibility to antiretroviral drugs. The IC_{50}s for the test recombinant virus were compared to those for a drug-susceptible reference HIV-1 strain (strain HXB-2). Viruses were categorized as sensitive or having reduced susceptibility on the basis of established drug-specific cutoff values. These values were based on the natural variations in the phenotypic susceptibilities of approximately 1,000 HIV-1 isolates from treatment-naïve patients. Values considered evidence of reduced susceptibility were increases in the IC_{50}s of more than 2.5-fold for saquinavir and amprenavir; 3-fold for stavudine, abacavir, and indinavir; 3.5-fold for didanosine, zalcitabine, and ritonavir; 4-fold for zidovudine, adefovir, and nelfinavir; 4.5-fold for lamivudine; 6-fold for efavirenz; 8-fold for nevirapine; and 10-fold for delavirdine. Data were also analyzed by use of the previously used assay cutoff value (fourfold change in the IC_{50}) (5).

The PhenoSense HIV assay was performed at ViroLogic Inc. as described by Petropoulos et al. (16). Briefly, HIV-1 PR- and RT-coding sequences were amplified by reverse transcription-PCR and cloned into a recombinant HIV vector containing a luciferase reporter gene. The constructs were transformed into *Escherichia coli* to produce resistance test vectors (RTVs). Viral stocks were prepared by cotransfecting cultures of cells of the 293 cell line with RTV DNA and an expression vector that produces the envelope proteins from an amphotropic murine leukemia virus. Pseudotyped virus particles were harvested from the transfected cell cultures and used to infect fresh 293 cells. Drug susceptibility was measured by adding PIs to transfected cells or RTIs to infected 293 cells; the levels of virus replication were measured on the basis of luciferase activity. Since RTVs are replication defective, luciferase activity is measured following a single round of replication. The IC_{50}s were compared to those for a drug-susceptible reference HIV-1 strain (strain NL4-3). Increases in the IC_{50}s of more than 2.5-fold were considered evidence of reduced susceptibility.

Genotypic analysis. The PR and RT sequences from the specimens were also determined from the patient-derived PCR products generated for the Antivirogram assay or from RTVs generated for the PhenoSense HIV assay by using the ABI Prism Dye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, Calif.). The sequences from the specimens were compared with those of the drug-susceptible reference strains, and all amino acid substitutions were reported by using the VircoGEN (Virco) or the GeneSeq HIV (ViroLogic Inc.) assay. Primary drug resistance-associated mutations (i.e., mutations that are selected by a drug and that confer resistance to that drug) were used to examine the association with phenotypic changes in some samples according to algorithms reported by Hirsch et al. (7). Two exceptions in the algorithms were made. The T215Y mutation in the presence of three or more zidovudine resistance mutations was considered a primary mutation for stavudine resistance (1, 8, 15), while the M184V mutation alone was not considered a primary mutation for abacavir resistance (4, 14).

RESULTS

Phenotypic drug susceptibility testing results were available for 42 specimens by the Antivirogram assay and 40 specimens by the PhenoSense HIV assay. The results available for specimens tested by both assays were compared. These included 529 pairs of results for 38 specimens whose recombinant virus isolates were tested for their susceptibilities to 12 to 15 drugs. Eighteen specimens were from recently seroconverted drug-naïve persons, and 20 were from patients who were the sources of occupational exposures, including 11 from drug-experienced individuals. Of the 529 pairs of results, 246 were for NRTIs and NtRTIs, 111 were for NNRTIs, and 172 were for PIs.

The overall concordance of the results and the distribution of concordant and discordant results with respect to drug and drug class are shown in Table 1. A high level of concordance (91.5%) was seen among the 529 pairs of results. Most of the concordant results (94.4%) were for the sensitive phenotype. The 45 (0.5%) discordant results were randomly distributed among 26 specimens. Twenty-three of the specimens with discordant results were from drug-experienced patients, while 22 of the specimens with discordant results were from drug-naïve persons. The proportion of discordant results differed by drug class. For the NRTIs-NtRTIs the numbers of specimens with concordant and discordant results were 230 (93.5%) and 16

TABLE 2. Distribution of 45 discordant results with respect to cutoff values for the Antivirogram or PhenoSense HIV assays

Fold[a]	No. (%) of results		
	AV-r and PS-s[b]	PS-r and AV-s[c]	Total
>1–2	12[d]	16[e]	28 (62.2)
>2–3	2	2	4 (8.9)
>3–4	2	5	7 (15.5)
>4–5	1	2	3 (6.7)
>5–6		1	1 (2.2)
>6–7		1	1 (2.2)
>7		1	1 (2.2)
Total	17	28	45

[a] Fold IC_{50}s above assay cutoff value.
[b] Reduced susceptibility by the Antivirogram assay but sensitive by the PhenoSense HIV assay.
[c] Reduced susceptibility by the PhenoSense HIV assay but sensitive by the Antivirogram assay.
[d] Distributed among 11 specimens.
[e] Distributed among 12 specimens.

(6.5%), respectivetly; for the NNRTIs the numbers were 101 (91%) and 10 (9%), respectively; for the PIs the numbers were 153 (89%) and 19 (11%), respectively. These findings suggest that discordance in results is slightly higher for PIs than for NNRTIs or NRTIs.

The PhenoSense HIV assay detected 474 sensitive and 55 reduced susceptibility phenotypes; 96.4 and 49.1% of these results, respectively, were confirmed by the Antivirogram assay. Conversely, Antivirogram detected 485 sensitive and 44 reduced susceptibility phenotypes; 94.2 and 61.4% of these results, respectively, were confirmed by PhenoSense HIV.

We also analyzed the distribution of discordant results by level of fold changes in IC_{50}s, and the data indicate that the discrepant results observed in our study predominantly involved isolates with low-level reduced susceptibility (Table 2). Most of the discordant results had values within twofold of the assay cutoff values and were distributed among 21 specimens. Of the 19 discordant results for PIs, 11 (57.9%) had fold differences of 1.55 or less. The highest fold difference seen was 18.5 for delavirdine in one specimen with discrepant results.

To better illustrate the types of discordant results, data for 11 specimens in which 27 discrepant results were observed are shown in Table 3. Both assays detected high-level resistance to all three drug classes in the recombinant virus isolates from these specimens (e.g., lamivudine resistance for specimens IV, V, and VI and both NNRTI and PI resistance for specimen I). The high-level resistance observed in the isolates from all these specimens was explained by the presence of primary resistance mutations. Among the isolates from specimens with discrepant

TABLE 3. Representative examples of discordant results

Specimen	Assay	Fold increase in IC_{50}[a]													
		AZT	3TC	d4T	ddC	ABC	ADV	NVP	DLV	EFV	SQV	IDV	RTV	NFV	AMP
I	PhenoSense HIV	7.35	4.71	**1.77**	1.17	1.94	1.48	>893	19.7	101.4	87.0	11.8	42.3	>536	3.06
	Antivirogram	22.3	7.3	**5.9**	1.7	2.7	2.8	>45	>110	283	>40	16.2	19.1	>41	5.1
II	PhenoSense HIV	0.73	**10.3**	0.83	2.00	**2.73**	0.64	0.89	1.52	0.66	0.78	0.57	0.56	0.92	0.61
	Antivirogram	1.3	**0.7**	1.8	0.4	**<0.4**	2.0	1.8	2.8	1.0	0.6	1.4	<0.2	1.0	0.6
III	PhenoSense HIV	**2.86**	1.77	1.47	1.7	0.96	1.40	**7.84**	**18.5**	**5.21**	0.74	0.74	0.97	1.07	0.57
	Antivirogram	**1.7**	0.4	2.1	3.3	1.0	0.5	**0.5**	**2.7**	**2.9**	0.3	1.0	0.3	0.7	1.0
IV	PhenoSense HIV	0.29	>183	0.84	**2.09**	**2.53**	0.45	0.59	1.00	0.60	0.93	0.78	0.80	0.69	0.61
	Antivirogram	0.8	>39	1.3	**3.9**	**0.6**	0.5	1.4	0.7	1.1	0.9	1.3	0.4	<0.2	1.3
V	PhenoSense HIV	0.48	>183	0.75	1.84	**2.90**	0.61	45.9	94.3	24.4	0.91	0.84	1.00	0.91	0.61
	Antivirogram	1.7	>38	0.4	1.3	**1.4**	0.6	>75	>160	322	1.3	0.8	0.3	0.7	0.6
VI	PhenoSense HIV	**5.47**	>183	1.29	**2.31**	**6.89**	1.42	0.96	0.66	0.71	1.22	0.88	1.01	0.80	0.89
	Antivirogram	**2.6**	35.5	1.0	**3.7**	**2.5**	0.8	1.2	0.5	0.7	0.4	0.9	0.2	0.5	0.9
VII	PhenoSense HIV	100.5	**4.70**	**2.67**	1.46	**5.17**	**2.96**	>625	1.05	**4.59**	**1.30**	1.23	**3.05**	2.58	0.80
	Antivirogram	13.5	**2.5**	**0.8**	0.6	**2.1**	**1.2**	52.3	0.4	**1.8**	**3.6**	1.4	**2.3**	4.6	1.8
VIII	PhenoSense HIV	1.03	1.40	0.86	1.0	1.01	0.88	**2.94**	2.03	**7.18**	0.84	0.88	1.13	1.21	1.03
	Antivirogram	2.1	1.2	0.7	2.1	0.8	0.6	**4.0**	3.7	**2.4**	0.6	1.3	0.4	0.4	1.2
IX	PhenoSense HIV	1.73	1.2	1.21	1.13	0.89	—[b]	**3.51**	—	1.86	0.67	0.92	0.83	1.07	—
	Antivirogram	1.2	1.2	0.7	0.6	<0.4	—	**7.5**	—	2.2	1.0	1.7	1.0	1.4	—
X	PhenoSense HIV	0.87	1.1	1.28	1.34	0.87	—	0.73	**2.91**	1.42	**1.11**	0.95	**2.3**	2.68	—
	Antivirogram	1.5	<0.3	0.3	1.2	<0.3	—	1.0	**6.4**	1.7	**4.0**	1.20	**3.7**	8.0	—
XI	PhenoSense HIV	0.48	0.94	0.72	0.85	0.89	0.95	83.5	8.22	5.34	**2.67**	1.96	3.0	3.29	1.39
	Antivirogram	1.8	1.2	1.0	1.6	0.4	2.6	>63	84.6	16.0	**2.4**	1.7	5.1	5.3	1.5

[a] Fold increase in IC_{50} compared to the IC_{50}s for the wild-type HIV-1 reference strains. Discordant results are highlighted in boldface. Not shown are phenotypic results for didanosine. All samples were sensitive to didanosine by both assays. See footnote *a* of Table 1 for drug abbreviations.
[b] —, paired results not available.

results, those from specimens II, IV, V, VI, and VII had low-level reduced susceptibility to abacavir by the PhenoSense HIV assay. All five specimens were from drug-experienced patients who were the sources of occupational exposures and who had been treated with lamivudine and other NRTIs but not with abacavir. The isolate from specimen II had a mixture of the wild-type M and the mutant V amino acid at codon 184, the isolates from specimens IV and V had the M184V mutation, and the isolate from specimen VI had the M184V and T215Y mutations. While these mutations or combinations are not known at present to be associated with abacavir resistance (4, 14, 20), the correlation between phenotype and genotype for abacavir may not be fully known. The isolate from specimen VII had D67N, L210W, and T215Y mutations, which could account for the reduced susceptibility of the isolate to abacavir (13). The evidence for the mixture of M and V in the isolate from specimen II could explain the finding of low-level reduced susceptibility to lamivudine by the PhenoSense HIV assay only.

The results for specimens III and VIII illustrate the discrepant results seen for NNRTIs. These specimens were from patients who were the sources of occupational exposures and who had no history of treatment with NNRTIs. The isolate from specimen III had an I135T mutation, which has been associated with low-level resistance to nevirapine and delavirdine in the PhenoSense HIV assay, with mean changes of 2.2- and 2.52-fold, respectively (11). However, the role of this polymorphism in the observed resistance to nevirapine and delavirdine in the isolate from specimen III is not known. The isolate from specimen VIII had no known NNRTI-associated primary resistance mutations.

We also analyzed the frequency of primary resistance mutations in the isolates from the 45 specimens with discordant results. We found that isolates from 37 (82.2%) specimens with discordant results have no known primary resistance mutations that would explain the reduced susceptibility seen in one of the two assays. Isolates from eight specimens with discrepant results (Table 3) had known primary resistance mutations and included isolates with reduced susceptibilities to the following: (i) zidovudine in the presence of M184V and zidovudine-associated mutations (specimen VI), (ii) lamivudine in the presence of a mixed M and V genotype at codon 184 (specimen II), (iii) stavudine in the presence of T215Y and other zidovudine resistance mutations (specimens I and VII), (iv) zalcitabine in the presence of the M184V mutation (specimens IV and VI), or (v) saquinavir in the presence of the L90M mutation(specimens X and XI).

The data were also analyzed by the cutoff values previously used for the Antivirogram assay (fourfold changes in IC_{50}s for all drugs). A concordance of 92.25% between the results of the Antivirogram and the PhenoSense HIV assays was seen. The level of discordance in the results observed in this analysis differed by drug class. The proportion of discordant results was higher for NNRTIs (14.4%) but lower for PIs (11%). The proportion of discordant results that had values within twofold of the assay cutoff values decreased from 62.2 to 53.6% compared to 62.2% observed in analysis with the presently used cutoff values.

DISCUSSION

HIV-1 drug resistance testing is increasingly becoming an important part of patient management. Phenotypic assays can play an important role in such testing because they can directly measure drug susceptibility. The commercial availability of improved high-throughput recombinant virus-based assays has made phenotypic testing feasible for patient management. To our knowledge, this study is the first one to compare the results obtained by the Antivirogram and the PhenoSense HIV assays, which differ in their testing strategies. We analyzed the data by phenotypic category, and a 91.5% correlation was seen between the results of these assays. We also found that the correlation for the results indicating reduced susceptibility was lower than that for the results indicating sensitivity. However, the values for the majority of the specimens with discordant results were found to be close to the assay cutoff values; the discordant results were not associated with primary resistance mutations and, therefore, are of unknown significance. The overall high degree of correlation was reassuring and indicated that both assays generate similar results, despite the use of different testing approaches.

Our study included specimens from both drug-naïve and drug-experienced patients. Interpretation of drug susceptibility category was based on cutoff values established independently for each assay to define what represents a drug susceptibility result significantly different from that for wild-type viruses. We observed that the discordance rate by phenotypic category was low (8.5%) and was not associated with drug experience since about half of the specimens with discordant results were from drug-naïve persons. However, in a few cases discordant results clustered within a specimen, suggesting that the recombinant virus populations evaluated in the two assays differed. This was obvious for patient VII, for whom all of the reductions in susceptibility values for the NRTIs and NNRTIs by the PhenoSense HIV assay were greater than those by the Antivirogram assay. The same might be true for patients II, III, and VI but was less obvious because the reduction in susceptibility was restricted to a single drug class.

We also found that the HIV-1 isolates with discordant results in general had no known primary resistance mutations that predicted high-level resistance. In addition, neither assay missed any of the specimens whose isolates had clear genotypic evidence of reduced susceptibility. The discordant results observed for specimens from drug-experienced patients were limited to a few observations related either to low-level cross-resistance, such as that for abacavir, or to the presence of a mixture of wild-type and drug-resistant genotypes, as seen in a specimen with isolates with M and V codons at position 184. The reduced ability of lamivudine-resistant virus to grow relative to that of wild-type virus might explain the difficulty in demonstrating a high-level lamivudine resistance in this specimen.

Our analysis of the distribution of the discrepant results according to the fold changes in the IC_{50}s also indicated that these discrepancies were frequently near the assay cutoff values for the definition of reduced drug susceptibility, suggesting that the clinician should evaluate both the absolute fold change in the IC_{50} and the categorization of "sensitive" or "reduced susceptibility" for a complete assessment of the test results.

Since clinically relevant cutoff values have not been established for most drugs, it is difficult at present to predict whether these discrepant results will have any clinical importance.

Analysis by either the presently or the previously used cutoff values for these assays showed no significant difference in the overall concordance of the results (91.5 versus 92.2%). However, some changes in the proportions of discordant results were seen, with an increase noted for PIs and a decrease noted for NNRTIs with the presently used cutoff values. These differences can be explained by the presently used cutoff values for the Antivirogram assay, which were reduced for three PIs and which were increased for all three NNRTIs.

The primary objective of the present study was to analyze the concordance of results by phenotypic category because of the direct implications for patient management. However, it may also be worthwhile to compare quantitatively the drug susceptibility levels obtained by each assay. This information may be important for the development of new antiretroviral drugs, in which phenotypic drug resistance testing is used extensively. For instance, in preclinical studies of a new candidate drug, assessment of phenotypic activity against well-characterized wild-type and resistant viruses is crucial. However, a proper quantitative comparison of the IC_{50}s obtained by different assays requires a large set of data of phenotypic testing results for viruses with diverse susceptibility profiles for each drug. Our study does not provide enough resistance datum points to compare IC_{50}s or fold changes in IC_{50}s between assays.

In conclusion, the present study indicates that, overall, the results of the Antivirogram and PhenoSense HIV assays correlate well, despite the use of different testing strategies. The data also indicate the importance of evaluating both the absolute fold change in the IC_{50} and the phenotypic category for a complete assessment of test results.

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